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PLASMA TO EGG CONVERSION FACTOR FOR EVALUATING POLYCHLORINATED BIPHENYL AND DDT EXPOSURES IN GREAT HORNED OWLS AND BALD EAGLES

KARL D. STRAUSE,† MATTHEW J. ZWIERNIK,*† SOOK HYEON IM,‡ JOHN L. NEWSTED,†§ DENISE P. KAY,§ PATRICK W. BRADLEY,† ALAN L. BLANKENSHIP,†§ LISA L. WILLIAMS,‖ and JOHN P. GIESY#††

†Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824, USA
‡DK Science Research & Development Center, DK B/D, 27-4 Gongduck-dong, Mapogu, Seoul, Korea
§ENTRIX Okemos, Michigan 48823, USA
‖U.S. Fish and Wildlife Service East Lansing Field Office, 2651 Coolidge Road, East Lansing, Michigan 48823
#Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, Special Administrative Region, China
††Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada

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Abstract—The benefits of nondestructive sampling techniques, such as plasma sampling, to directly measure contaminant exposure levels in at-risk or protected raptor populations are many. However, such assays are generally inconsistent with the most certain source of toxicity reference values, which are based on feeding studies and quantified as dietary or ''in ovo'' (egg-based) concentrations. An accurate conversion factor to translate nondestructive plasma-based contaminant concentrations to comparable egg-based concentrations will prove valuable to risk assessors investigating the potential effects of chemical exposures to raptors. We used databases describing the concentrations of total polychlorinated biphenyls (PCBs) in great horned owls (GHO; *Bubo virginianus*) and total PCBs and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in bald eagles (*Haliaeetus leucocephalus*) from the Great Lakes region (Michigan, Wisconsin, USA) to develop a relationship to predict concentrations of PCBs and DDE in eggs. To develop a robust predictive relationship, all of the source data included concentrations of both total PCBs and/or DDE for nestling blood plasma and egg samples collected from within discrete active nesting territories and, in most instances, the same nest. The key characteristics (slope and elevation) of each relationship were tested for differences related to species and geographic region. Predicted variability of relationships were examined and compared to variability associated with natural systems. The results of statistical testing indicate that applying the conversion factors between species (GHO to bald eagle) and among geographic regions yields predicted egg concentrations that are not statistically dissimilar and are within the natural variability observed for residue concentrations among eggs of raptors within species and region.

Keywords—Raptors Polychlorinated biphenyls Plasma Egg Nondestructive sampling

INTRODUCTION

Because raptors are at the top of the food chain, they are maximally exposed to many persistent and bioaccumulative residues [1]. This, combined with the fact that they are susceptible to the toxic effects of many contaminants of concern, means that raptors can be used as effective and sensitive biological monitors for contaminant exposures and assessment of environmental effects [2] (www.ncrs.fs.fed.us/epubs/owl/SHEFFIE.PDF). Raptors also are often used as environmental sentinels for monitoring of contaminants [3] or as primary or surrogate receptor species in ecological risk assessments [4] (www.dnr.state.wi.us/ORG/water/wm/foxriver/documents/ra/Final%20BLRA%20Section%201.pdf). Raptors are particularly useful, because they are often territorial and long lived, reproducing in the same territory over long periods of time. Thus, extensive databases of historical contaminant exposures are often available.

Historically, contaminant monitoring programs utilizing raptors have primarily used eggs because of the several advantages of using them for assessing contaminant exposure and effects. These include ease of collection and the fact that the proximal exposure of the developing embryo to the chemicals gives a direct measure of one of the most sensitive endpoints, embryo lethality [5]. Eggs are relatively easy to transport and store, and egg samples from wild bird populations are available independent of egg fertility. In addition, since lipophilic compounds tend to accumulate in lipids of eggs, quantification of residues is facilitated. Furthermore, controlled laboratory studies, including feeding and egg-injection studies, offer direct comparisons of concentrations of residues in eggs with effects such as survival, eggshell integrity, and developmental deformities [6–9]. Egg-based contaminant exposure measurements have also been correlated with temporal and spatial effects. Nevertheless, egg sampling has some serious limitations when used in site-specific and long-term investigations of potential ecological risk. These include the destructive nature of the sample, a high level of nest disturbance that significantly increases the frequency of nest abandonment, high levels of uncertainty for assigning spatial origin to the observed exposure concentration, and narrow temporal limits to the ''window'' of monitored exposure ([10,11]; R. Frank, 1997, Master's thesis, University of Wisconsin, Madison, WI, USA). Egg sampling efforts also may be limited by the gender-restricted nature of the sample.

When the disadvantages of determining egg-based exposure data outweigh the advantages, residues are measured in blood plasma [12–14]. This approach also has several advantages.

* To whom correspondence may be addressed (zwiernik@msu.edu).

These include the ability to collect blood without destroying the individual, the ability to collect samples from the same individual over time, and the ability to collect samples from nestlings [14]. Because nestlings are sedentary and most residues in their blood are accumulated from food; nestling blood plasma is an integrated measure of concentrations of residues in the area proximal to the nest site, much more so than are concentrations of residues in eggs or adult plasma samples [11,15]. For most raptor species, collection of blood plasma from nestlings reduces the risk of injury to the bird and minimizes abandonment or nest relocation by adult birds. Also, blood samples need not be gender or age specific. Use of blood plasma has been further advanced by development of more sensitive methods of residue analyses that has lessened the mass of analyte required for quantification.

Limitations of plasma contaminant data are primarily in interpreting the effects of residues in blood plasma. There is relatively little information relating the concentrations of specific residues in blood plasma of nestling raptors to adverse outcomes, while there is more information on the effects of concentrations of residues in eggs on effects on both individuals and populations. Long-term monitoring of residues in eggs, blood, and raptor populations has demonstrated that trends in concentrations of residues are similar for eggs and blood plasma [15]. Thus, the use of blood plasma for monitoring populations for adverse effects would be facilitated by predicting concentrations of residues in blood to concentrations in eggs.

We used synoptic sampling of blood plasma from nestling great horned owls (GHO; *Bubo virginianus*) and bald eagles (*Haliaeetus leucocephalus*) from the Great Lakes region to develop a relationship to predict concentrations of polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in eggs. We compared these relationships to those previously published for bald eagles from other regional subpopulations and assessed the variability of predicting total PCB concentrations in eggs from those in blood plasma.

MATERIALS AND METHODS

Collection of great horned owl blood plasma and eggs

Blood plasma from nestlings and fresh or addled eggs of GHO were collected from the Kalamazoo River Superfund Site (KRSS). Collections were made between April 2000 and April 2004 along a 190-km stretch of the river's floodplain between the cities of Marshall and Saugatuck, Michigan, USA (Fig. 1). Collections were made from both naturally occurring nests and artificial nesting platforms. This location represented a gradient of concentrations of both PCBs and ΣDDT (dichlorodiphenyltrichloroethane [DDT] and its metabolites DDE and dichlorodiphenyldichloroethane [DDD]) that ranged from local ''background concentrations'' to relatively great concentrations of PCBs and ΣDDT [16]. Matched egg and nestling plasma samples were collected from nests of the same mated pair occupying the same nest in the same reproductive year. In other instances, matched egg and nestling plasma were collected from the same nest over a period of two or more years. In cases where nesting pairs selected a new nest site, samples were collected from alternate nests within the same territory over two or more consecutive years. To encourage renesting after collection of eggs, fresh eggs were collected as soon as possible following confirmation of incubation. Addled eggs were obtained when nests were abandoned.

Eggs were labeled, transported back to the laboratory, and stored at 4°C until processing. Length, width, and whole-egg weight and water volume were measured prior to removal of contents. Egg contents were stored in solvent-rinsed glass jars at −20°C until measurement of PCBs and ΣDDT.

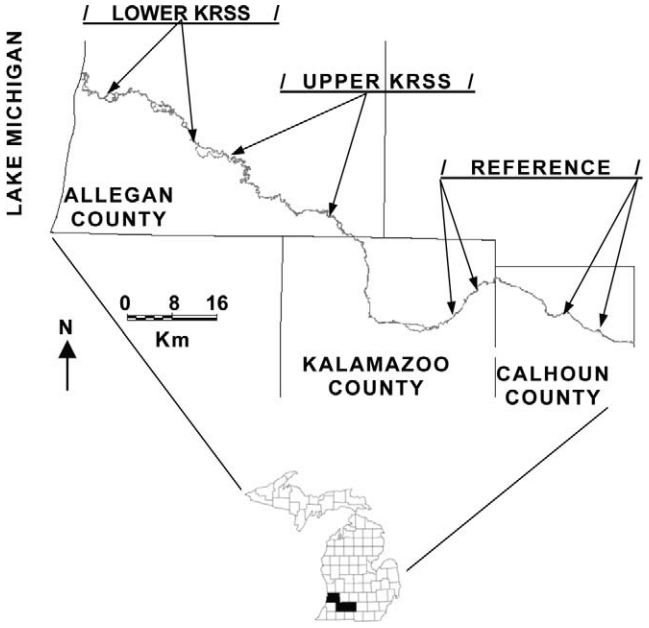

Fig. 1. Map of area of the Kalamazoo River superfund site (KRSS), indicating the location in southern Michigan, USA, as well as the three reaches across which a gradient of polychlorinated biphenyl (PCB) concentrations was observed.

Nestling blood samples were collected when nestlings were approximately 4 to 6 weeks of age and had attained a minimum body weight of 0.75 kg. A sample of 5 to 7 ml was withdrawn from the brachial vein with a heparinized disposable syringe (25-gauge hypodermic needle) and sterile technique. Blood was transferred to a heparinized Vacutainer™ (BD, Franklin Lakes, NJ, USA) tubes and labeled. Vacutainers containing whole blood were centrifuged at 1,200 rpm for 10 min within 48 h of field sampling. Plasma (supernatant) was transferred to a new Vacutainer appropriately labeled and stored upright at −20°C until measurement of PCBs and ΣDDT.

Whole egg homogenates and nestling plasma samples were processed and analyzed for congener-specific total PCBs and ΣDDT using methods described previously [17]. All chemical concentrations in eggs were corrected for moisture loss [18].

Collection of bald eagle blood plasma and eggs

The values used to develop the egg to plasma relationships for bald eagles in the Great Lakes region were compiled from studies conducted by several state and federal agencies as well as public and private research institutes, the majority of which were completed between 1996 and 2002. With a few exceptions, most egg samples included in this database originated from the U.S. Fish and Wildlife Service (USFWS) environmental contaminants program using addled egg collection [19]. Most measurements of residues in blood plasma were from the Michigan wildlife contaminant trend monitoring program administered by the Michigan Department of Environmental Quality (MDEQ), Office of Surface Water Quality [20–23] (web links for these references are located at the following web links, respectively: (1) www.deq.state.mi.us/documents/deq-wd-swqas-wildlifetrendreport02.pdf, (2) www.deq.state.mi.us/documents/deq-wd-swqas-wildlifetrendreport.pdf, (3) www.

deq.state.mi.us/documents/deq-wd-swqas-2000eaglereport.pdf, and (4) www.deq.state.mi.us/documents/deq-wd-swq-gleas-99baldeaglereport.pdf). Additional data were also used ([17,24,25]; W. Bowerman, 1991, Master's thesis, Northern Michigan University, Marquette, MI, USA).

All studies reported concentrations of total PCB, and/or *p,p′*-DDE for blood plasma of nestlings and/or egg samples collected from discrete active eagle nesting territories. From these reports, concentrations of individually paired blood plasma and egg samples were assembled according to the following three general guidelines: First, plasma samples collected from 1996 forward were paired with egg samples collected within a 5-year window of sampling for the two media (e.g., egg [1997] paired with plasma [2001]); second, samples collected prior to 1996 were paired only for the same or two consecutive collection years; third, for either grouping, a third or fourth sample was included in instances where two consecutive collections of plasma or egg were made (e.g., egg [1986–1987], plasma [1987–1988]), in which case the geometric mean concentration of the two combined samples was used. The selection of a 5-year maximum window for pairing the most recent samples was based on the trend monitoring data for PCB and *p,p′*-DDE concentrations in eggs that were not significantly different within subpopulations, between years from 1996 onward [19].

Sample collection and processing for these studies are consistent with the methods described for Kalamazoo River GHO, but methods of chemical analyses varied to some degree. The USFWS analyses of *p,p′*-DDE and total PCBs in addled eggs were completed by the USFWS Patuxent Analytical Control Facility (Shepherdstown, MD, USA) using gas-liquid chromatography. Nominal lower limits of detection were 10 ng/g, (wet wt) for DDE and 50 ng/g for total PCBs. Egg concentrations were corrected for moisture loss [19]. The MDEQ analyses of *p,p′*-DDE and total PCBs (sum of 20 PCB congeners) in nestling plasma were completed at the Clemson Institute of Environmental Toxicology (Clemson, SC, USA) using capillary gas chromatography with electron capture device following U.S. Environmental Protection Agency approved methods [23]. All reported results were confirmed by dual column analyses. Quantification levels for both compounds were 2 ng/g [20–23]. For use in this assessment, the MDEQ PCB plasma concentrations for the 20 quantified PCB congeners were converted to a total PCB equivalent using the relationship: total PCBs = 4.57(sum 20 PCB congeners, ng/g, wet wt) + 0.98 [24]. Analyses of *p,p′*-DDE and total PCBs in nestling plasma for the Green Bay and Fox River samples [25] were completed at Michigan State University and included the use of gas chromatography with electron capture detection and confirmation with mass spectrometry. Detection limits were 2.5 ng/g for DDE and 5 ng/g for total PCBs. Analytical methods for additional egg and plasma samples from Green Bay and Fox River [26] are provided by the authors.

Statistical analyses

Sample sets were analyzed for normality by the Kolmogorov–Smirnov, one-sample test with Lilliefors transformation. Concentration data were log-normally distributed and after log-transformation satisfied assumptions of normality. To evaluate the plasma to egg relationship for each PCB and *p,p′*-DDE database, a Pearson product-moment correlation analysis was performed on the log-transformed values. Paired blood plasma and egg concentrations of residues were plotted as a function of the blood plasma values and the line of best fit for each sample set was derived through simple regression and residuals analyses. Normality and correlation analyses were completed using the Statistica (Ver 6.1) statistical package (Statsoft, Tulsa, OK, USA). Regression residuals were calculated using Excel (Microsoft® Windows PE, 2002; Microsoft, Redmond, WA, USA).

To assess the robustness of the relationships developed for the GHO, predictions were compared with measured values for bald eagles at other locations available in the literature. Tests for homogeneity of regression coefficients and elevation used analysis of covariance methods (ANCOVA) [27]. Multiple comparisons among elevations were made by use of Tukey's honestly significant difference (HSD) for unequal sample size [27]. The criterion for significance used in all tests was $p < 0.05$. Comparisons of conversion factor predictive variability were made by calculating the relative percent difference (RPD).

To test the similarity between egg to nestling plasma relationships between bald eagles of the Pacific Coast versus bald eagles of the Great Lakes, an ANCOVA was used to test for equality of the population regression coefficients (slope) and elevation. Tests of elevation may be considered to be the same as asking whether the two population *y* intercepts are different. However, one must be cautious of misleading interpretations from such a characterization if discussion of the *y* intercepts would require a risky extrapolation of the regression lines far below the range of *x* for which data were obtained [27,28]. The use of elevations instead of *y* intercepts also assures comparison of relationships over only the range of plasma or egg concentrations measured in each study and eliminates the potentially confounding effects that analytical detection limits may contribute to a test of *y* intercepts. If either test identified a statistically significant difference within the pool of data being evaluated, additional pair-wise comparisons were completed using Tukey's HSD to identify which of the population slopes or elevations differed from one another.

RESULTS

Great horned owls

The fidelity of GHO to established home territories and preferred nesting sites resulted in multiple instances of renesting that provided samples of both nestling blood plasma and eggs for the same breeding pair in the same nesting territory. A total of 14 paired GHO nestling blood plasma and egg samples were assembled from a total of 16 blood plasma and 17 egg samples. This included four nests where the samples of blood plasma and eggs were collected in the same nest during the same year and three nests where the paired samples were collected from the same nest but during different years. In seven instances, samples were collected from two different nests within an active nesting territory but during different years.

Relationships between concentrations of both total PCBs and ΣDDT were investigated. Total PCB concentrations for these 14 data points (Table 1) exhibited a gradient among the three reaches of the KRSS and there was a significant positive correlation ($r = 0.766$, $p = 0.001$) between log-normalized nestling blood plasma and egg PCB concentrations (Fig. 2). The narrow range of ΣDDT concentrations detected in KRSS GHO plasma and egg did not exhibit such a gradient and concentrations of ΣDDT in eggs were negatively correlated

Table 1. Great Lakes (Kalamazoo River) great horned owl plasma to egg polychlorinated biphenyl (PCB) conversion factor sample pairing

PCB sample location[a] (sample year: plasma/egg)	Plasma (ng PCB/ml)	Egg (μg PCB/g) (wet wt)
Lower KRSS (2002/2000)	147	12.2
Lower KRSS (2002/2001)	147	19.8
Lower KRSS (2002/2002)	147	25.7
Lower KRSS (2002/2004)	147	2.09
Upper KRSS (2002/2003)	80.4	1.61
Upper KRSS (2000/2002)	60.2	2.74
Upper KRSS (2001/2003)	43.7	1.61
Upper KRSS (2003/2003)	31.3	0.61
Lower KRSS (2003/2004)	31.0	2.78
Reference (2003/2001)	25.9	0.31
Reference (2003/2002)	25.9	0.17
Reference (2003/2003)	25.9	0.22
Upper KRSS (2001/2002)	24.1	2.74
Reference (2002/2002)	14.0	0.21

[a] Kalamazoo River superfund site, Kalamazoo, Michigan, USA.

with those in blood plasma ($r = -0.735$, $p = 0.003$). Thus, a ΣDDT egg to blood plasma predictive relationship was not developed for GHO.

Great Lakes bald eagles

A total of 30 (total PCBs) and 31 (*p,p′*-DDE) paired nestling plasma and egg samples were assembled from the available individual samples from the Great Lakes region (Table 2). Pairings originate from a single nesting territory and are combined following the pairing guidelines discussed previously. Great Lakes bald eagles exhibited significant positive correlations between total PCB and *p,p′*-DDE concentrations in nestling blood plasma and egg samples (PCBs: $r = 0.789$, $p < 0.001$; *p,p′*-DDE: $r = 0.569$, $p = 0.001$). Log-normalized PCB and *p,p′*-DDE concentrations were plotted along with the line of best fit and 95% confidence interval for the regression line (Figs. 3 and 4).

Confirmation of concept

To test if the generality of relationships for the prediction of concentrations of residues in eggs from those in blood plasma of nestlings are consistent between owls and eagles, and among geographically distinct subpopulations, ANCOVA for slope and elevation were conducted. The relationship between residue concentrations in nestling blood plasma and egg has not been previously investigated for GHO, but Elliott and Norstrom [13] and Elliot and Harris [29] have examined the distribution of PCBs and *p,p′*-DDE in bald eagle nestling plasma and egg samples from the Pacific Coast of Canada and the United States. We have presented a comparison of the key characteristics of each of the four PCB databases (one GHO, three bald eagle) and three bald eagle *p,p′*-DDE databases for which nestling plasma and egg contaminant relationships are now available (Tables 3 and 4). A more robust egg to blood plasma relationship is obtained by using individual samples from the same nest or a much larger number of summary mean concentrations [29].

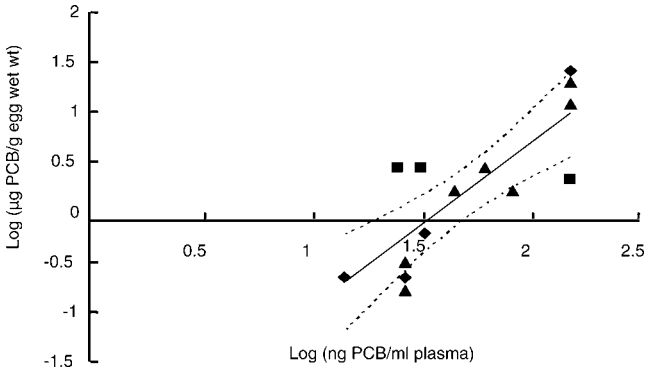

Fig. 2. Concentration of total polychlorinated biphenyls (PCBs) in eggs of great horned owls as a function of PCBs in blood plasma of nestling great horned owls along the Kalamazoo River, Michigan, USA. ◆ = same nest/same year; ■ = same nest/different year; ▲ = same territory/different year. Regression line with 95% confidence intervals of the predicted line: $[\log_{10}(\mu g\ PCB_{egg}/g, \text{ wet wt}) = 1.647[\log_{10}(\text{ng } PCB_{plasma}/ml)] - 2.578$ ($p < 0.001$, $r^2 = 0.666$).

There were no significant differences between the slopes of regression lines for concentrations of PCB in eggs and nestling blood plasma (ANCOVA, $p > 0.05$) for the three bald eagle groups. A graphical representation of the PCB and *p,p′*-DDE egg to nestling blood plasma relationships between the four PCB sample groups and three *p,p′*-DDE sample groups is provided (Figs. 5 and 6). There were some statistically significant differences among elevations for these three groups (ANCOVA, $p < 0.003$). The elevation of the Great Lakes bald eagle group was significantly different from the two Pacific Coast groups (Tukey's test, $p < 0.03$). These findings indicate that the three population samples are all estimates of the common population regression coefficient and are approximately parallel, but with differing elevations and differing values for a predicted *y* (egg concentration). The slope of the relationship for the GHO from the Kalamazoo River was not different from that of the bald eagles (ANCOVA, $p < 0.05$), but the elevation was significantly different (Tukey's test, $p < 0.001$). The elevation of the relationship for GHO was significantly different from that of the two Pacific Coast bald eagle groups (Tukey's test, $p < 0.03$) but not significantly different (Tukey's test, $p < 0.05$) than the Great Lakes bald eagle group. Taken together, the results of the ANCOVA and Tukey's tests of differences in elevation for the PCB egg to plasma relationship indicate that the observed differences among the four groups is unlikely to be related to differences between species. For the *p,p′*-DDE data set, there were no statistically significant differences among either slopes or elevations of the relationship between concentrations in eggs and nestling blood plasma for the three bald eagle groups (ANCOVA, $p > 0.05$). This indicates that the three sample groups could have been drawn at random as subpopulations from the same population.

Egg predictions

To examine the predictive variability of results obtained from the various relationships, measured nestling blood plasma concentrations from the Great Lakes bald eagle database were used in each of the four PCB conversion factor equations and three DDE conversion factor equations to predict concentrations of PCBs and DDE in eggs. The predicted PCB–DDE egg concentration was then compared to the measured egg PCB–DDE concentration comprising the plasma–egg pair from the same Great Lakes bald eagle database. This approach allowed predictions made from each relationship to be compared to measured PCB–DDE concentrations in eggs. Differences between the predicted and measured egg PCB–DDE concentrations were assessed by calculating the RPD between the two values: $\text{RPD} = [|\text{measured}_{egg} - \text{predicted}_{egg}|/(\text{measured}_{egg} + \text{predicted}_{egg})/2] \cdot 100$. The analysis was conducted with three

Table 2. Great Lakes (Michigan, Wisconsin, USA) bald eagle plasma to egg polychlorinated biphenyl (PCB) and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) conversion factor sample pairing

PCB sample location (sample year: plasma/egg)	Plasma (ng PCB/ml)	Egg (μg PCB/g) (wet wt)	Plasma (ng DDE/ml)	Egg (μg DDE/g) (wet wt)	*p,p′*-DDE sample location (sample year: plasma/egg)
Peshtigo River (1992/1992)[ab]	901	66.6	361	14.7	Peshtigo River (1992/1992)[ab]
10 Mile Creek (1999/1997)[acd]	866	8.0	83.0	2.90	10 Mile Creek (1999/1997)[ac]
10 Mile Creek (1999/1999)[acd]	866	9.0	83.0	2.60	10 Mile Creek (1999/1999)[ac]
North Lake (1999–2000/1999)[aed]	856	3.30	114	5.20	North Lake (1999–2000/1999)[af]
Ottawa Marsh (2000/2000)[g]	773	42.2	27.0	8.10	Ottawa Marsh (2000/2000)[ae]
Skull/Stoney Island (1999/1996)[acd]	394	12.0	15.0	4.90	Skull/Stoney Island (1999/1996)[ac]
Swan Creek (1999/1996)[acd]	367	41.2	14.0	8.19	Swan Creek (1999/1996)[ac]
Boutlier Lake (1987/1986)[ah]	319	55.3	235	29.9	Boutlier Lake (1987/1986)[ah]
Dinsmoore (2002/1999)[aid]	281	6.16	39.0	4.0	Dinsmoore (2001/1999)[ai]
Pere Marquette River (2000–2001/2002)[g]	235	23.9	31.0	14.9	Pere Marquette River (2000/2002)[eg]
Pere Marquette River (2000–2001/1998)[ag]	235	34.0	31.0	0.29	Pere Marquette River (2000/1998)[ae]
Upper Peninsula Vulcan (2002/1999)[aid]	206	13.0	5.0	15.0	Upper Peninsula Vulcan (2002/1999)[ai]
Oxbox Lake (2000/2000)[aed]	176	3.40	4.0	0.72	Oxbox Lake (2000/2000)[ae]
Badwater Lake (2002/2000)[aid]	175	1.50	7.0	0.30	Badwater Lake (2002/2000)[ai]
Big Charity Island (1999/1996)[acd]	162	18.9	9.0	3.74	Big Charity Island (1999/1996)[ac]
Upper Peninsula Vulcan (1999/1999)[acd]	146	13.0	5.0	15.0	Upper Peninsula Vulcan (1999/1999)[ac]
Copper Peak (2000/1996)[ade]	106	4.30	14.0	1.30	Copper Peak (2000/1996)[ae]
Badwater Lake (1999–2000/2000)[ade]	52.0	1.50	4.0	0.30	Badwater Lake (1999–2000/2000)[ae]
Huron Bay (1999–2000/2000)[ade]	45.0	1.45	5.0	0.91	Huron Bay (1999–2000/2000)[ae]
Peavy Pond East (1999–2000/2000)[ade]	41.0	2.20	12.0	1.30	Peavy Pond East (1999–2000/2000)[ae]
Iron Lake (2002/2000)[adi]	34.0	0.65	9.0	0.62	Iron Lake (2002/2000)[ai]
Fortune Lake Island (2000/2000)[ade]	28.0	0.47	10.0	0.51	Fortune Lake Island (2000/2000)[ae]
Paint Lake (2002/2000)[adi]	23.0	0.48	18.0	0.26	Paint Lake (2002/2000)[ai]
Rock Lake/Carney Outlet (2002/1999)[adi]	22.0	1.10	10.0	0.10	Rock Lake/Carney Outlet (2002/1999)[ai]
Boney Falls (2000–2001/1999)[adef]	16.0	1.90	3.0	0.60	Boney Falls (2000–2001/1999)[aef]
Boney Falls (2000–2001/2000)[adef]	16.0	2.20	3.0	0.93	Boney Falls (2000–2001/2000)[aef]
Buck/Armstrong Lake (2001/1997)[adf]	14.0	0.55	5.0	0.62	Iron Lake (1999–2000/2000)[ae]
Fox River (1991–1992/1990)[j]	195	36.0	4.0	0.38	Thousand Isle Lake (1999–2000/2000)[ae]
Big Creek (2000/2001)[g]	43.0	0.98	3.0	0.62	Black Creek Flooding (1999/1997)[ac]
Otter Lake (2000–2001/2000)[adef]	13.0	0.49	3.0	0.10	Michigamme River (1999/1998)[ac]
			3.0	0.44	Blue Lakes (1999/1997)[ac]

[a] Egg concentrations from United States Fish and Wildlife Service [19].
[b] Plasma and egg concentrations from Dykstra et al. [25].
[c] Plasma concentrations from Michigan Department of Environmental Quality [23].
[d] PCB plasma concentrations for 20 PCB congeners are converted to a total PCB equivalent using the relationship: total PCBs = 4.57(sum 20 PCB congeners, ng/g) + 0.98 [26].
[e] Plasma concentrations from Michigan Department of Environmental Quality [22].
[f] Plasma concentrations from Michigan Department of Environmental Quality [21].
[g] Plasma and/or egg concentrations from Strause [17].
[h] Plasma concentrations from Bowerman, 1991 (Master's thesis, Northern Michigan University, Marquette, MI, USA).
[i] Plasma concentrations from Michigan Department of Environmental Quality [20].
[j] Plasma and egg concentrations from Dykstra and Meyer [24].

randomly selected subsamples from the Great Lakes eagle database. Using a random number generator, three sets of 10 paired plasma–egg samples were selected from the 30 PCB samples and 31 DDE samples comprising the Great Lakes bald eagle database.

A second and more restrictive evaluation included calculating the mean predicted versus measured egg RPD for a subset of the Great Lakes eagle plasma–egg samples with a restricted range of plasma concentrations that matches or falls within the range of plasma concentrations used to establish each specific egg to plasma relationship.

Predictive variability was assessed in each of the three randomly selected subsets and the single restricted subset. Results of the RPD analysis are expressed as mean RPD. (Tables 5 and 6). For the randomly selected subsets and PCB relationships, predicted egg PCB concentrations from the Great Lakes eagle database produced the lowest range of mean RPD (73–78%), which would be expected since this relationship was derived from the same plasma and egg samples used in the comparison. Using the GHO conversion factor, mean PCB RPD values ranged from 74 to 97%. Mean PCB RPD values for the two Pacific Coast eagle conversion factors had the widest ranges from 73 to 97% and 70 to 100%. Predictive variability for the restricted PCB subsets shows that the GHO conversion factor produced the lowest mean RPD (55%) among the four PCB conversion factors. For *p,p′*-DDE, the predicted egg concentrations from the Great Lakes eagle database again produced the lowest range of mean RPD (52–81%) for the randomly selected subsets and the restricted sample (77%), as expected. Mean DDE RPD ranges for the two Pacific Coast eagle conversion factors included 89 to 93% and 95 to 97%, and restricted mean RPDs were 99% and 105%.

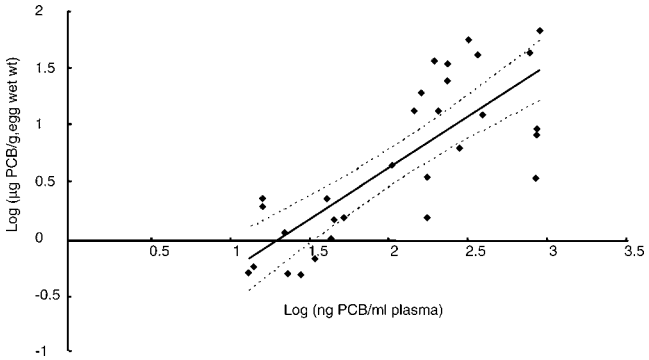

Fig. 3. Concentration of total polychlorinated biphenyls (PCBs) in eggs of bald eagles as a function of PCBs in blood plasma of nestling bald eagles in the Great Lakes region (Michigan, Wisconsin, USA). Regression line with 95% confidence intervals of the predicted line: $\log_{10}$ (µg PCB_{egg}/g, wet wt) = 0.905[$\log_{10}$ (ng PCB_{plasma}/ml)] − 1.193 ($p < 0.001$, $r^2 = 0.623$).

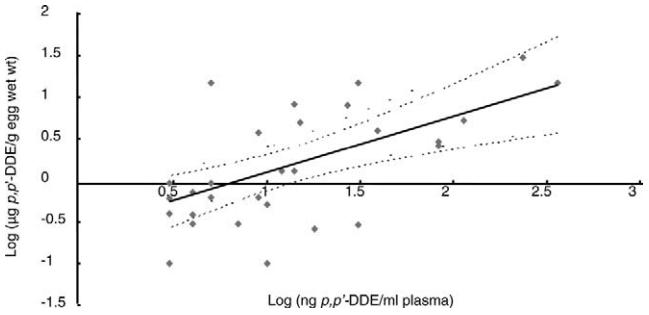

Fig. 4. Concentration of total *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in eggs of bald eagles as a function of *p,p′*-DDE in blood plasma of nestling bald eagles in the Great Lakes region (Michigan, Wisconsin, USA). Regression line with 95% confidence intervals of the predicted line: $\log_{10}$ (µg *p,p′*-DDE_{egg}/g, wet wt) = 0.676[$\log_{10}$ (ng *p,p′*-DDE_{plasma}/ml)] − 0.578 ($p < 0.001$, $r^2 = 0.324$).

DISCUSSION

Because nestlings can be captured and handled more easily than adults, it is less likely to cause harm to individuals or the population [13,14]. The use of blood plasma from nestlings eliminates the potentially confounding influence of adult exposures during migrations or on their wintering grounds or in instances where resident, nonmigratory species or individuals shift or greatly expand winter foraging territories [30]. Because most of the residues in the blood plasma of nestlings is accumulated from the area proximate to the nest, these results can be more easily compared with the results of diet studies to identify significant contributing sources of environmental contaminants in the food web [31]. Also, because the embryo and nestling are the most sensitive life stages for population-level effects of chlorinated hydrocarbon contaminants, field monitoring of nestling plasma combined with laboratory feeding and egg injection studies often provides the best opportunities to integrate laboratory and field studies in efforts to derive and verify field-based toxicity reference values for plasma-based endpoints [32,33].

Predictive accuracy of the plasma to egg conversion factor

Focused studies to specifically examine the predictive accuracy of the plasma to egg relationship are not available in the literature. Nevertheless, the relationship has been used in several studies as a tool for further interpretation of adult and nestling plasma data. The earliest efforts to predict concentrations of a residue in eggs from measurements in blood plasma was for ΣDDT concentrations in adult falcons and accipiter hawks. In wild American kestrels (*Falco sparverius*) of the Pacific Northwest, ΣDDT residues in adult female plasma closely paralleled ΣDDT residues in eggs laid by the same birds [34]. Concentrations of DDE in eggs (lipid basis) corresponded well with concentrations of DDE in adult European sparrow hawks (*Accipiter nisus*) [35]. A significant decrease in total concentrations of DDE in the bodies of females due to translocation to eggs was also observed. In both laboratory and field studies, concentrations of DDE in blood plasma of American kestrels correlated with exposure in the diet. Concentrations of ΣDDT in adult female blood plasma in populations of three accipiters (goshawk [*A. gentilis*], Cooper's hawk [*A. cooperii*], sharp-shinned hawk [*A. striatus*]) were correlated with ΣDDT concentrations in eggs [12]. Those authors also described a large decline in female kestrel plasma

Table 3. Plasma to egg conversion factors (CF) for total polychlorinated biphenyls (PCBs), Orders Strigiformes and Falconiformes

		PCB Plasma to Egg Relationships					
Species (subpopulation)	Reference	Sample pairing (*n*)	Strength[a]	Plasma sample range (ng PCB/ml)	Egg sample range (µg PCB/g) (wet wt)	Slope[b]	Intercept[b]
Great horned owl (Great Lakes)	[17]	Individual pairs[c] (14)	$r^2 = 0.666$ $p < 0.001$	14–147	0.2–25.7	1.647	−2.578
Bald eagle (Great Lakes)	[17]	Individual pairs[c] (30)	$r^2 = 0.623$ $p < 0.001$	13–901	0.47–66.6	0.905	−1.193
Bald eagle (Pacific Coast)	[13]	Geometric mean pairs[d] (6)	$r^2 = 0.869$ $p < 0.03$	14–80	2.6–12.7	0.734	−0.409
Bald eagle (Pacific Coast/Great Lakes)	[29]	Geometric mean pairs[d] (9)	$r^2 = 0.785$ $p = 0.001$	14–207	2.4–31.3	0.824	−0.583

[a] r^2 and significance values for the line of best fit. Values for CF Equations [13,29] describe the database as it is presented in each respective manuscript. All remaining values are derived from the databases and analyses included in this assessment.
[b] Regression slope and *y*-intercept values presented in Table 3 describe a log-log relationship between nestling plasma total PCB concentration (x = ng/ml) and fresh or addled whole egg total PCB concentration (y = µg/g wet wt).
[c] Individual nestling plasma and egg sample pairings representative of exposure conditions within an active raptor nesting territory.
[d] Geometric mean nestling plasma and egg sample pairings representative of exposure conditions within a regional raptor breeding area.

Table 4. Plasma to egg conversion factors (CF) for *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), Order Falconiformes

Species (subpopulation)	Reference	*p,p′*-DDE plasma to egg relationships					
		Sample pairing (*n*)	Strength[a]	Plasma sample range (ng DDE/ml)	Egg sample range (μg DDE/g) (wet wt)	Slope[b]	Intercept[b]
Bald eagle (Great Lakes)	[17]	Individual pairs[c] (31)	$r^2 = 0.324$ $p < 0.001$	3–361	0.1–29.9	0.676	−0.578
Bald eagle (Pacific Coast)	[13]	Geometric mean pairs[d] (6)	$r^2 = 0.912$ $p < 0.01$	7–100	2.2–9.7	0.637	−0.220
Bald eagle (Pacific Coast/Great Lakes)	[29]	Geometric mean pairs[d] (9)	$r^2 = 0.918$ $p < 0.001$	3–100	1.0–9.7	0.680	−0.318

[a] r^2 and significance values for the line of best fit. Values for conversion factor equations [13,29] describe the database as it is presented in each respective manuscript. All remaining values are derived from the databases and analyses included in this assessment.
[b] Regression slope and *y*-intercept values presented in Table 4 describe a log-log relationship between nestling plasma total PCB concentration (x = ng/ml) and fresh or addled whole egg total PCB concentration (y = μg/g wet wt).
[c] Individual nestling plasma and egg sample pairings representative of exposure conditions within an active raptor nesting territory.
[d] Geometric mean nestling plasma and egg sample pairings representative of exposure conditions within a regional raptor breeding area.

ΣDDT concentrations due to egg laying or transfer to eggs, similar to that observed for sparrow hawks. Studies of ΣDDT exposure were conducted during a period when researchers were investigating the egg-thinning effects of DDT and its metabolites DDD and DDE, and the maternal transfer of contaminates to eggs was of primary interest to researchers who were deciphering the mechanisms of action for this compound. Separate adult plasma (female) to egg conversion factors for ΣDDT were developed for wild, nesting kestrels (Falconidae) and accipiter hawks (Accipitridae) [12]. The parameters describing the two relationships were not statistically different for the two species. Therefore, those authors suggested use of a pooled regression to predict egg concentrations from concentrations of DDT in adult blood plasma for these two families. Use of the pooled data set in the log-log relationship for the two species resulted in a relationship that could be used to make comparisons among species. While these relationships provide useful background information, the DDT relationships were not compared to the relationships developed in this paper because they did not meet the selection criteria in the present study.

The pooled relationship developed for kestrel and accipiter hawks in one region [12] has been used to predict concentrations of ΣDDT and PCBs in eggs from concentrations in blood plasma from wintering adult eagles in Colorado and Missouri, USA [36]. The pooled relationship was used to predict concentrations of ΣDDT in eggs from plasma and to assess the potential for DDE-caused eggshell thinning and potential impacts to reproductive success of individual eagles. The egg to adult plasma ΣDDT relationship also was used to estimate the potential hazard to reproductive success of migrating peregrine falcons exposed to DDE on their wintering grounds [37]. The same relationship was used to predict concentrations of DDE in egg from concentrations of DDE in blood plasma of adult bald eagles so that the exposure of those eagles could be compared to addled eggs collected from the same subregion [38]. In that study predicted concentrations of DDE in eggs corresponded very well with measured values (RPDs <10%). However, concentrations of DDE in blood plasma were predicted from concentrations of DDE in whole blood that had been adjusted for loss of DDE during storage and dilution. The relationship developed for kestrels and accipiter hawks also was used in two separate studies to predict concentrations of ΣDDT in eggs of several South African raptors, including the

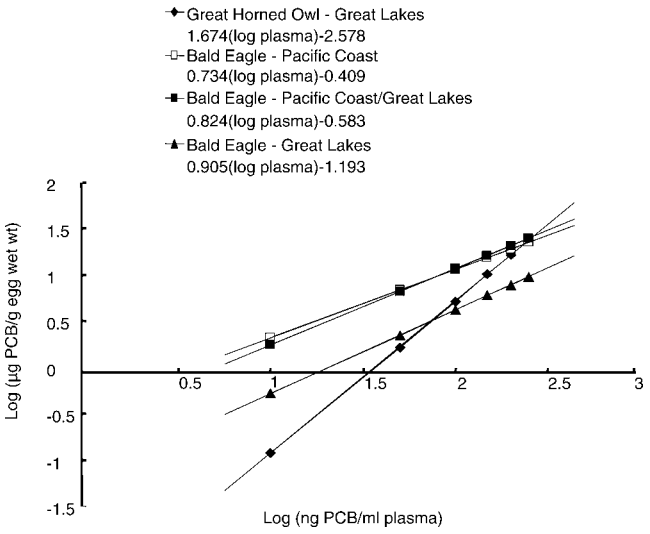

Fig. 5. Concentration of total polychlorinated biphenyls (PCBs) in eggs of great horned owls bald eagles as a function of PCBs in blood plasma of nestlings, of the same species, respectively. Regression lines are plotted and predictive equations are given in the figure legend.

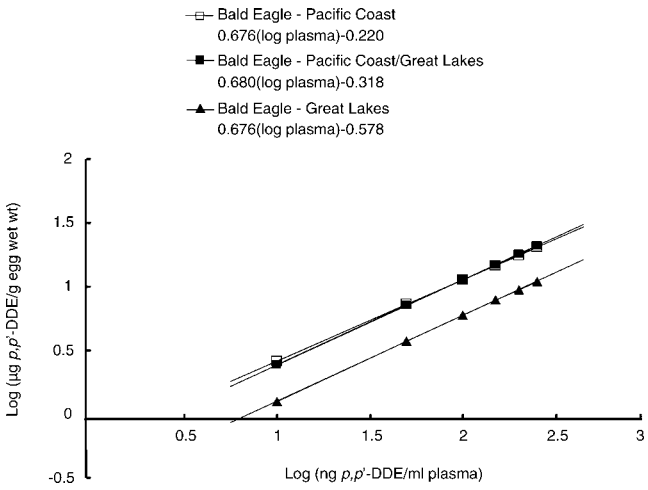

Fig. 6. Concentration of *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in eggs of bald eagles as a function of *p,p′*-DDE in blood plasma of nestlings, of the same species, respectively. Regression lines are plotted and predictive equations are given in the figure legend.

Table 5. Bald eagle and great horned owl (GHO) relative percent difference[a] (RPD) assessment for predicted versus measured egg polychlorinated biphenyl (PCB) concentrations across three randomly selected[b] cohorts (n = 10) and the plasma-restricted[c] cohort drawn from the Great Lakes bald eagle database[d]

Predicted vs measured PCB concentrations		Mean predicted RPD (%) from measured			
		Pacific Coast bald eagle [13]	Pacific Coast/Great Lakes bald eagle [29]	Great Lakes GHO [17]	Great Lakes bald eagle[e] [17]
Random sample no.1		100	97	93	77
Plasma range (ng PCB/ml)	16–866				
Egg range (μg PCB/g wet wt)	1–55				
Random sample no.2		92	90	74	73
Plasma range (ng PCB/ml)	16–773				
Egg range (μg PCB/g wet wt)	0.5–55				
Random sample no.3		70	73	83	78
Plasma range (ng PCB/ml)	106–866				
Egg range (μg PCB/g wet wt)	4–42				
Plasma-restricted sample		117	97	55	74
Plasma range (ng PCB/ml)		14–52 ng/ml	14–206 ng/ml	14–146 ng/ml	13–901 ng/ml
Egg range (μg PCB/g wet wt)		0.5–2.2 μg/g	0.5–362 μg/g	0.5–13 μg/g	0.5–67 μg/g
n		11	18	13	30

[a] RPD = $\{|Y_P - Y_M|/[(Y_P + Y_M)/2]\}\cdot(100)$. Y_P = predicted egg, Y_M= measured egg.
[b] Selected subsample identified using random number generator to select n = 10 plasma/egg paired samples from n = 30 (PCB) and n = 31 (DDE) possible individual paired data points in the Great Lakes bald eagle database.
[c] Plasma-restricted subsample includes all individual paired data points in the Great Lakes bald eagle database lying within the range of plasma concentrations used to derive each specific PCB and *p,p′*-DDE conversion factor databases examined in this manuscript. Sample sizes specific to the plasma-restricted subsample are noted below.
[d] See Table 2 for individually paired plasma and egg data.
[e] Great Lakes bald eagle database included for comparison purposes, illustrating effects of sample variability and site-specific exposure potentials on the intrinsic predictive accuracy of the line of best fit across the source database.

greater kestrel (*F. repicoloides*) and the lanner falcon (*F. biarmicus*), and pied kingfishers (*Ceryle rudis*). In both studies a screening-level hazard assessment was conducted by comparing the predicted concentration of ΣDDT to a toxicity reference value based on concentrations of DDE in the eggs of sensitive raptor and piscivorous species [39–42]. More recently, the USFWS and MDEQ [26] derived toxicity reference values based on concentrations of PCBs and DDE in blood plasma of nestling bald eagles using plasma to egg relationships [29] and field-based benchmarks (concentrations in eggs that were associated with reduced productivity) [43,44].

The major obstacle to assessing the predictive accuracy of the plasma to egg relationship is the absence of an extensive database containing individually matched plasma and egg con-

Table 6. Bald eagle relative percent difference[a] (RPD) assessment for predicted versus measured egg *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) concentrations across three randomly selected cohorts[b] (n = 10) and the plasma-restricted cohort[c] drawn from the Great Lakes bald eagle database[d]

Predicted vs measured *p,p′*-DDE concentrations		Mean predicted RPD (%) from measured		
		Pacific Coast bald eagle [13]	Pacific Coast/Great Lakes bald eagle [29]	Great Lakes bald eagle[e] [17]
Random sample no.1		95	89	81
Plasma range (ng/ml)	3–39			
Egg range (μg/g wet wt)	0.1–15			
Random sample no.2		99	93	52
Plasma range (ng/ml)	3–361			
Egg range (μg/g wet wt)	0.1–15			
Random sample no.3		97	93	66
Plasma range (ng/ml)	4–361			
Egg range (μg/g wet wt)	0.3–15			
Plasma-restricted sample		105	99	77
Plasma range (ng DDE/ml)		7–83 ng/ml	3–83 ng/ml	3–361 ng/ml
Egg range (μg DDE/g wet wt)		0.1–15 μg/g	0.1–15 μg/g	0.1–30 μg/g
n		16	28	31

[a] RPD = $\{|Y_P - Y_M|/[(Y_P + Y_M)/2]\}\cdot(100)$. Y_P = predicted egg; Y_M= measured egg.
[b] Randomly selected subsample identified using random number generator to select of n = 10 plasma/egg paired samples from n = 30 (PCB) and n = 31 (DDE) possible individual paired data points in the Great Lakes bald eagle database.
[c] Plasma-restricted subsample includes all individual paired data points in the Great Lakes bald eagle database lying within the range of plasma concentrations used to derive each specific PCB and *p,p′*-DDE conversion factor databases examined in this manuscript. Sample sizes for the plasma-restricted subsample are noted below.
[d] See Table 2 for individually paired plasma and egg data.
[e] Great Lakes bald eagle database included for comparison purposes, illustrating effects of sample variability and site-specific exposure potentials on the intrinsic predictive accuracy of the line of best fit across the source database.

Table 7. Estimated polychlorinated biphenyl (PCB) concentration in egg (±95% PI)[a] from a relevant range of field-based nestling exposure or plasma concentrations

	Predicted egg concentrations (μg PCB/g wet wt) total PCB exposure[b]							
	Individual paired samples				Geometric mean paired samples			
Plasma concentration (ng PCB/ml)	Great Lakes great horned owl		Great Lakes bald eagle		Pacific Coast bald eagle		Pacific Coast/Great Lakes bald eagle	
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
	1.674	−2.578	0.905	−1.193	0.734	−0.409	0.824	−0.583
10	0.12 (0.01–1.49)		0.52 (0.06–4.57)		2.11 (0.83–5.39)		1.74 (0.58–5.25)	
50	1.66 (0.17–15.8)		2.22 (0.27–17.9)		6.89 (2.98–16.0)		6.56 (2.46–17.5)	
100	5.20 (0.51–52.4)		4.15 (0.52–33.0)		*11.5 (4.28–30.8)*		11.6 (4.17–32.4)	
150	10.1 (0.92–111)		5.99 (0.75–47.7)		*15.43 (5.12–46.7)*		16.2 (5.53–47.6)	
200	*16.3 (1.36–193)*		7.77 (0.97–62.2)		*19.1 (5.76–63.4)*		20.6 (6.7–63.2)	
250	*23.5 (1.83–300)*		9.51 (1.18–76.5)		*22.4 (6.28–80.7)*		*24.7 (7.73–79.1)*	

[a] Upper and lower 95% prediction interval (PI) for predicted egg *y*. Predicted egg concentrations (±95% PIs) for plasma values lying beyond the range of data used to derive each respective conversion factor are in italics.
[b] Regression slope and *y*-intercept values describe a log-log relationship for total PCB concentrations in plasma (ng PCB/ml) and egg (μg PCB/g wet wt).

centrations from the same nest. The database assembled from the USFWS addled egg monitoring [19] and the MDEQ plasma sampling [20–23] that is presented here provided a basis for this evaluation. The predictive variability RPD results indicate that applying the conversion factor between species (GHO to bald eagle) and among geographic regions (Pacific Coast to Great Lakes) yielded predicted egg concentrations that are within the natural variability observed for residue concentrations among eggs of raptors within a species and region. Within-clutch RPD values for total organochlorine pesticide concentrations in nonmigratory sparrow hawks in Great Britain have been observed to be as great as 32%, while mean concentration (clutch means) between-clutch RPD values from the same female on the same territory are as great as 63% [45]. Relative percent difference values as great as 171% for PCBs (3-year interval) and 80% for DDE (4-year interval) have been seen for sparrow hawks from a subpopulation of females nesting within the same geographic subregion [46]. Studies of PCB and DDE concentrations in eggs sampled from the same population of eagles nesting in the vicinity of Green Bay exhibited between-clutch variability that ranged from 38% (1-year interval) to 125% (4-year interval) RPD for PCBs, and from 31% (1-year interval) to 133% (4-year interval) RPD for *p,p′*-DDE [43].

Utility and application

There is variability inherent in predicting concentrations of PCBs and *p,p′*-DDE in eggs using the egg to plasma relationships for GHO and bald eagle presented in the present assessment. The predicted values and widely bounded 95% prediction intervals for a relevant range of nestling blood plasma concentrations illustrate the homogeneity of slopes and heterogeneity of elevations among the four PCB and three DDE relationships (Tables 7 and 8). The predicted PCB and DDE egg concentrations for the Pacific Coast bald eagles show a consistent difference from the Great Lakes eagle and GHO predicted values. Even at their greatest, the observed divergences represent only a three-fold difference in the range of predicted egg concentrations that would be significant for an ecological risk assessment (e.g., at or above the toxicity reference value threshold concentration). This indicates that for ecological risk assessment applications, the use of a plasma

Table 8. Estimated *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) concentrations (±95% PI)[a] in egg from a relevant range of field-based nestling exposure/plasma concentrations

	Predicted egg concentrations (μg DDE/g wet wt) *p,p′*-DDE exposure[b]					
			Geometric mean paired samples			
Plasma concentration (ng DDE/ml)	Individual pairs Great Lakes bald eagle		Pacific Coast bald eagle		Pacific Coast/Great Lakes bald eagle	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
	0.676	−0.578	0.637	−0.220	0.668	−0.282
10	1.26 (0.08–20.0)		2.61 (1.29–5.29)		2.43 (1.34–4.41)	
50	3.72 (0.22–61.9)		7.28 (3.52–15.0)		7.13 (3.86–13.2)	
100	5.94 (0.34–105)		11.3 (5.03–24.4)		11.3 (5.85–21.9)	
150	7.81 (0.42–144)		*14.7 (6.1–35.1)*		*14.9 (7.39–29.8)*	
200	9.48 (0.50–180)		*17.6 (6.95–44.4)*		*18.0 (8.70–37.2)*	
250	11.0 (0.56–216)		*20.3 (7.68–53.4)*		*20.9 (9.86–44.2)*	

[a] Upper and lower 95% prediction interval for predicted egg *y*. Predicted egg concentrations (±95% PIs) for plasma values lying beyond the range of data used to derive each respective conversion factor are in italics.
[b] Regression slope and *y* intercept values describe a log:log relationship for *p,p′*-DDE concentrations in plasma (ng DDE/ml) and egg (μg DDE/g, wet wt).

to egg conversion factor would introduce minimal levels of uncertainty to calculations of risk.

CONCLUSION

Birds will continue to be used as indicators of environmental contamination due to their ubiquitous global distributions, high metabolic rates, and diverse foraging habits. The advantages that bird eggs provide as a medium for assessing the bioavailability of lipophilic contaminants will undoubtedly encourage the selection of egg-based sampling programs and toxicity reference values will continue to be based on concentrations of residues in eggs. To minimize effects on populations and maximize the site-specific assessment of exposures in some cases, measurement of residues in blood plasma will be more practical.

We have demonstrated that concentrations of residues in blood plasma can be used to predict concentrations of persistent and bioaccumulative compounds in eggs by use of a blood plasma to egg conversion factor. The egg to plasma relationships derived herein from individually paired great horned owl and bald eagle samples in Great Lakes populations are not statistically dissimilar from comparable egg to plasma relationships provided in the literature for Pacific Coast bald eagles. These findings also indicate that raptors express similar relationships between nestling plasma and egg concentrations across closely related avian orders and across geographically isolated subpopulations. The plasma to egg conversion factor can be used as an accurate and reliable tool to translate nondestructive plasma-based contaminant exposure measurements to comparable egg-based concentrations. These calculated egg-basis concentrations can then be used with egg-based toxicity reference values derived from population-level benchmark effects (e.g., embryo mortality, developmental deformities, fledgling productivity) to assess the health of animal communities. The plasma to egg conversion factor also offers ecological risk assessors an additional tool to aid with interpretations of dissimilar data. It is our hope that by incorporating plasma-based sampling protocols into long-term monitoring plans and site-specific hazard assessments that this method will contribute to more efficient and less disruptive studies of raptor populations in all habitats.

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